FIELD MODIFICATION FORM
FOR
LOWER PASSAIC RIVER RESTORATION PROJECT
THE LOUIS BERGER GROUP, INC.

DATE: December 23, 2009

DOCUMENT: Oversight Quality Assurance Project Plan (QAPP) for Biological Sampling, Community Surveys, and Toxicity and Bioaccumulation Testing
Lower Passaic River Restoration Project

ACTIVITY: QAPP Field Modification No. 3 for the Oversight Program

REQUESTED MODIFICATION:
According to the Lower Passaic River Restoration Project "Oversight Quality Assurance Project Plan (QAPP) for Biological Sampling, Community Surveys, and Toxicity and Bioaccumulation Testing" (Malcolm Pirnie, Inc., August 2009 and associated addenda), real-time modifications to the oversight project can be implemented by documenting the modification and obtaining approval from the Project Manager and Site Quality Officer (refer to Worksheet #6). Requested modifications for "Field Modification No. 3" include (1) the replacement of Standard Operating Procedures (SOPs) for the *Hyalella azteca*, *Ampelisca abdita*, and *Chironomus dilutus* toxicity tests and (2) documentation of the October 21, 2009 consensus decisions between the United States Environmental Protection Agency (USEPA) and the Cooperating Parties Group (CPG) to minimize variability between the toxicity tests conducted by the CPG laboratory and the USEPA laboratory. These modifications amend the following worksheets in the manner described below. Worksheets will be updated in the next submittal of the Oversight QAPP.

- Worksheet #9: On October 21, 2009, the USEPA, USEPA's consultant (The Louis Berger Group, as subcontractor to Malcolm Pirnie, Inc.), the government split sample laboratory (American Aquatic Testing, Inc.), the CPG, the CPG's consultant (Windward Environmental LLC), and the CPG's laboratory (EnviroSystems, Inc.) discussed the split sample toxicity testing program. The objective was to minimize variables that could impact survival and growth of test organisms in the CPG exposures and the corresponding government split sample exposures. The following consensus decisions were agreed:
  - EnviroSystems, Inc. and American Aquatic Testing, Inc. will purchase test organisms (*A. abdita* and *H. azteca*) from the same supplier (ARO, Inc. of Hampton, New Hampshire) to assure that test organisms used by both laboratories share a common culture history. *H. azteca* test organisms will include both freshwater individuals and individuals acclimated to 10 parts per thousand (ppt) salinity.
  - EnviroSystems, Inc. and American Aquatic Testing, Inc. will purchase *C. dilutus* from the same supplier (ABS, Inc. of Fort Collins, Colorado) to assure that test organisms used by both laboratories share a common culture history.
  - American Aquatics Testing, Inc. will collect the seawater and artificial substrate for all control exposures from ARO, Inc., which is also supplying material to EnviroSystems, Inc.

- American Aquatic Testing, Inc. will conduct one control sample with the artificial substrate for *H. azteca* and *A. abdita*.
- A standard reference toxicity test will be conducted on all test organisms regardless of transportation route to American Aquatic Testing, Inc.
- Laboratories will create their own freshwater, starting with de-ionized water. This consensus decision was superseded on November 23, 2009 when EnviroSystems, Inc. shipped freshwater to American Aquatic Testing, Inc.

- Worksheet #19, Footnote 4 and Worksheet 23, Footnote 2: Per USEPA request, sediment will ***not*** be sieved prior to the toxicity testing. While sieving is typically used to remove predators and debris from a sample, sieving can alter the grain-size distribution of the sample, which can consequently alter the contaminants present during the toxicity test.
- Worksheet #23: The CPG Remedial Investigation / Feasibility Study (RI/FS) QAPP was revised to include an updated *H. azteca* toxicity test (SOP QA-1467, version 07g, dated October 22, 2009). The attached revised CPG SOP for *H. azteca* supersedes the SOP (version 06) previously provided in the Oversight QAPP. Split sample toxicity testing will be conducted using the attached revised CPG SOP, with the following modification[1]:
  - SOP Section 2.0 describes a site-specific reference material; however, according to the CPG RI/FS QAPP Worksheet #9 (page 33), no site-specific reference material will be analyzed.
  - SOP Section 5.1.2: Project site sediment will be stored at 2-4 degrees Celsius (°C). The sample container will not be purged with inert gas once opened.
  - SOP Section 5.1.5: Overlying water quality (*i.e*., freshwater versus saline water) will be consistent with exposures conducted by EnviroSystems, Inc. Porewater salinity values will not be reported by American Aquatic Testing, Inc. for split sample exposures.
  - SOP Section 5.2: American Aquatic Testing, Inc. will receive all test organisms from ARO, Inc., the same supplier to EnviroSystems, Inc.
  - SOP Section 5.3.2: American Aquatic Testing, Inc. will receive artificial substrate from ARO. Inc. and will conduct one control sample.
  - SOP Section 5.4.2: Samples will not be sieved prior to the toxicity test.
  - SOP Section 5.4.3: American Aquatic Testing, Inc. will receive seawater from ARO, Inc. (the same supplier to EnviroSystems, Inc.). Freshwater will consist of a 50:50 (by volume) mix of natural water and re-constituted hard water that was created by American Aquatic Testing, Inc. using de-ionized water.
  - SOP Section 5.4.6: American Aquatic Testing, Inc. will not report porewater measurements.
- Worksheet #23: The CPG RI/FS QAPP was revised to include an updated *C. dilutus* toxicity test (SOP QA-1407, version 12c, dated November 18, 2009). The attached revised CPG SOP for *C. dilutus* supersedes the SOP (version 12) previously provided in the Oversight QAPP. Split sample toxicity testing will be conducted using the attached revised CPG SOP, with the following modification[2]:
  - SOP Section 5.1.2: Project site sediment will be stored at 2-4 °C. The sample container will not be purged with inert gas once opened.
  - SOP Section 5.2: American Aquatic Testing, Inc. will receive all test organisms from ARO, Inc., the same supplier to EnviroSystems, Inc.

[1] All split sample *H. axteca* toxicity tests were conducted following the CPG SOP Version 07g.
[2] All split sample *C. dilutus* toxicity tests were conducted following the CPG SOP Version 12c.

- SOP Section 5.3.6: American Aquatic Testing, Inc. will receive artificial substrate from ARO. Inc. and will conduct one control sample.
- SOP Section 5.3.7: American Aquatic Testing, Inc. will receive freshwater from EnviroSystems, Inc.
- SOP Sections 5.1 and 5.4.6: American Aquatic Testing, Inc. will not report water characteristic measurements.

- Worksheet #23: The CPG RI/FS QAPP was revised to include an updated *A. abdita* toxicity test (SOP QA-1426, version 08c, dated November 19, 2009). The attached revised CPG SOP for *A. abdita* supersedes the SOP (version 08) previously provided in the Oversight QAPP. Split sample toxicity testing conducted after November 11, 2009 will use the attached revised CPG SOP, with the following SOP modification[3]:
  - SOP Section 5.1.2: Project site sediment will be stored at 2-4 °C. The sample container will not be purged with inert gas once opened.
  - SOP Section 5.2: American Aquatic Testing, Inc. will receive all test organisms from ARO, Inc., the same supplier to EnviroSystems, Inc.
  - SOP Section 5.3.2: American Aquatic Testing, Inc. will receive seawater from ARO, Inc. (the same supplier to EnviroSystems, Inc.).
  - SOP Section 5.3.3: American Aquatic Testing, Inc. will receive artificial substrate from ARO. Inc. and will conduct one control sample.
  - SOP Sections 5.1 and 5.4.6: American Aquatic Testing, Inc. will not report water characteristic measurements.

RATIONALE:
SOPs for *H. azteca, A. abdita*, and *C. dilutus* toxicity tests are being replaced to be consistent with the CPG RI/FS QAPP. SOPs are being further modified to minimize variability between the toxicity tests conducted by the CPG-laboratory and the USEPA-laboratory.

ATTACHMENTS:
SOP QA-1467 (version 07g, dated October 22, 2009) prepared by EnviroSystems, Inc.
SOP QA-1407 (version 12c, dated November 18, 2009) prepared by EnviroSystems, Inc.
SOP QA-1426 (version 08c, dated November 19, 2009) prepared by EnviroSystems, Inc.

Len Warner
The Louis Berger Group, Inc. Project Manager: _____

AmyMarie Accardi-Dey
The Louis Berger Group, Inc.
Site Quality Control Officer Designee: _____

[3] *A. abdita* toxicity tests were prepared on November 3, 2009. Test organisms were placed in the test chamber on November 5, 2009 following SOP QA-1426, version 08a. The CPG provided the revised SOP (version 08b) to USEPA on November 10, 2009. Version 08c was provided on November 19, 2009.

TITLE: Assessment Toxicity (28-Day) of Sediments To The Amphipod, *Hyalella azteca* based on Survival and Growth -Project Specific Document. Rev. 6

Approved By:

QA Officer:_____ Date:_____

President:_____ Date:_____

Revision History:

Revision Number	Changes	Revised By	Date
0	Preparation of SOP	K. A. Simon	6/00
1	Review and update. Correction of light intensity	K. A. Simon	4/01
2	Update, NELAC revisions	K.A. Simon	07/01
3	Clerical Corrections clarifications related to water sources and sieving	K.A. Simon	09/01
4	Review and Update, Addition of NELAC Requirements	S. Dionne	03/02
5	Review and Update	K. A. Simon	04/04
6	Review and Update	K. A. Simon	07/06
7	Review and Update	R. A. McIsaac	01/09
7a	Project Specific Document	K. A. Simon	08/09
7b	Project Specific Document Rev 1	K. A. Simon	09/09
7c	Project Specific Document Rev 2. Includes modification suggest during 09/15/09 teleconference with Chris Ingersoll, USGS	K. A. Simon	09/09
7d	Project Specific Document Rev 3. Includes modification suggest during 09/28/09 teleconference with Chris Ingersoll, USGS	K. A. Simon	09/29/09
7e	Addition of pore water ammonia monitoring at the end of the assay. Rev 4	K. A. Simon	10/05/09
7f	Final EPA suggested changes to text. Rev 5	K. A. Simon	10/18/09
7g	Modification of Pore Water Monitoring Parameters in ¶ 5.4.6.3 and 11.21. Rev 6	K. A. Simon	10/22/09

1.0 Purpose and Applicability

The purpose of this Standard Operating Procedure is to determine the impact, based on survival and growth, of sediments to amphipods exposed under static renewal conditions. The assay involves exposing amphipods to a sediment over a 28 day period. The assay is conducted using guidelines developed by ASTM and is provided in *Standard Test Methods for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates* (ASTM E1706-05e).

Hyalella azteca (Saussure), Amphipoda, have many desirable characteristics of an ideal sediment toxicity testing organism including: relative sensitivity to contaminants associated with sediment, short generation time, contact with sediment, ease of culture in the laboratory, and tolerance to varying physico-chemical characteristics of sediment.

At the end of the 28 day exposure period the amphipods are recovered and counted to establish survival, then dried to establish growth expressed as the average weight/surviving individual and average biomass (total biomass in a replicate divided by the number of organisms in that replicate at the start of the exposure).

This document has been modified to meet project work scope requirements, specified by the U.S. Environmental Protection Agency, for the Lower Passaic River Ecological Risk Assessment. The work is being conducted under contract to Windward Environmental, LLC. Modifications incorporated into the document are related to the salinity of the overlying water used during the assay and culture and acclimation of the test organisms. These modifications are being made to allow the use of a single test species over an extended range of the project where salinity regimes vary beyond the normal range utilized for the species.

2.0 Definitions

Overlying Water: The water placed over sediment in a test chamber during a test.

Reference Sediment: A whole sediment near an area of concern used to assess sediment conditions exclusive of material(s) of interest. The reference sediment may be used as an indicator of localized sediment conditions exclusive of the specific pollutant input of concern. Such sediment would be collected near the site of concern and would represent the background conditions resulting from any localized pollutant inputs as well as global pollutant input. This is the manner in which reference sediment is used in dredge material evaluations.

Reference-Toxicity Test: A test conducted with reagent-grade reference chemical to assess the sensitivity of the test organisms. Deviations outside an established normal range may indicate a change in the sensitivity of the test organism population. Reference-toxicity tests are most often performed in the absence of sediment.

Pore water: Water located in spaces between grains of sediment.

Sediment: Particulate material that usually lies below water. Formulated particulate material that is intended to lie below water in a test.

Whole Sediment: Sediment and associated pore water which have had minimal manipulation. The term bulk sediment has been used synonymously with whole sediment.

3.0 Applicable Documents/References

ASTM. 2009. *Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates.* E 1706-05e, West Conshohocken, PA.

U.S. EPA. 2000. *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates.* Second Edition. EPA/R-99/064. March 2000.

ESI SOP# QA-1203-R5-2003: Preparation of Daphnia Food

ESI SOP#QA-1339-R4-2008: Collection of Sediment Pore Water Samples

ESI SOP#QA-1219-R0-2009: Use and Operation of the YSI Model 556 Multi Probe System meter

ESI SOP#QA-1114-R3-2003: Conduct of Reference Toxicant Assays

ESI SOP#QA-1341-R1-2009: Sulfide Analysis by Titration

ESI SOP#QA-1320-R6-2009: Statistical Analysis of Acute and Chronic Exposure Bioassay Data

ESI SOP#QA-1309-R4-2009: Computation of Hardness by Calculation Method

ESI SOP#QA-1326-R6-2009: Alkalinity by Lachat using the Automated Phenate Method

ESI SOP#QA-1325-R8-2009: Ammonia by Lachat

ESI SOP#QA-1336-R4-2007: Measurement of Total Organic Carbon using the Phoenix 8000 Analyzer

4.0 Materials and Apparatus

Test animals
Beakers, 400 mL, drilled and screened for flow through
Incubator/water bath capable of maintaining a temperature of 23 ±1°C
Dissolved oxygen meter, pH meter, conductivity meter, temperature logger
Light Meter
Balance, capable of reading 0.01 mg
Drying Oven, 60°C
Components for artificial sediment - fine sand, organic material
Sieves
YCT Food (See SOP #1203)
Refractometer

5.0 Methods/Procedures

5.1 Test Material

5.1.1 Test substances will be clearly identified as to source, collection date, period of collection and description of collection methods. The laboratory will identify sample storage procedures and maintain receipt, storage, usage and disposition records.

5.1.2 Project site sediments will be stored at 2-4°C in the original shipping container.

Maximum holding time is 8 weeks. Once open, a sample container with remaining sample will be purged using an inert gas prior to storage. Inert gas will be either nitrogen or argon.

5.1.3 Prior to test preparation, the sediment is warmed to 23°C.

5.1.4 Prior to use, sediment total organic content (loss on ignition) is measured for each sediment sample.

5.1.5 Prior to use in the assay, pore water salinity will be measured using a refractometer or salinometer, as appropriate. Samples having a pore water salinity of ≤5‰ will be identified as being tested using freshwater as the overlying water while all samples having a pore water salinity of >5‰ will be identified as being tested using a 10‰ salinity overlying water.

5.2 Test Organisms

5.2.1 Healthy amphipods from the same source and age are used in the test. Amphipods will be between 7 and 8 days old.

5.2.2 Confirmation of species is provided by the supplier. If not provided, ESI will use the services of either the zoology department of the University of New Hampshire or the taxonomy group of Normandeau Associates, Bedford, NH to confirm the species. Organisms maintained by ESI will be from cultures of the confirmed species.

5.2.3 Pretest observation data concerning the source, handling procedures, disease treatment (if any), health, feeding, mortality and mean dry weight of test animals will be recorded and reported. Mean dry weight of organisms at the start of the assay are based on that of 40 organisms measured in groups of 10. Growth, dry weight, of similar aged adult amphipods will be monitored in both freshwater and saltwater. Acclimated amphipod cultures will be monitored prior to use in the assays to provide a qualitative measurement of growth in the two culture systems. Young production in all cultures will be qualitatively compared to determine if there are substantial differences in reproduction between freshwater cultures and saltwater-acclimated cultures.

5.2.4 Organisms used in the assays will be selected from cultures of appropriate salinity. Salinity levels utilized will be either freshwater, <0.5‰, or 10‰, depending on the pore water salinity of an individual sample.

5.3 Exposure Conditions

Exposure conditions and monitoring requirements detailed in the following paragraphs are the minimum requirements specified by ESI for this assay.

5.3.1 Sediments are placed in vessels, overlying water is added and the samples are allowed to settle overnight before organisms are added to the test.

5.3.2 A laboratory control will be established for both fresh and salt water sample sets.

5.3.3 Assays are conducted in a static renewal mode. Overlying water is renewed on a

daily basis, at the rate of two (2) volume additions per day.

5.3.4 Mean water temperature is 23 ±1°C. Maximum temperature deviations should not exceed ±3°C of the specified value at any time.

5.3.5 The photoperiod is set to 16 hours light : 8 hours dark. Light intensity is 100 to 1000 lux from wide spectrum fluorescent fixtures.

5.3.6 The test vessels are 400 mL beakers containing 100 mL of sediment and 225 mL of water.

5.3.7 Sediment for the laboratory control treatment will be a formulated sediment. Formulated sediments will be prepared, by weight, as follows:

Fine grained sand	-	95%
Organic matter	-	5% (approximate)

Sand used will be an equal mix of F-75 and F-65 unground quartz (silica) sand provided by New England Silica

The preferred source for organic matter in the artificial sediment is the organic matter recovered from either midge larvae or amphipod cultures. The material is screened to remove large matter and then autoclaved for a half hour to insure sterility. Organic content of the control sediment will be adjusted prior to the start of the assay to be representative of that of the project site sediments.

5.3.8 The type of the overlying water will be determined based on individual sample pore salinity. Samples with a pore water salinity of ≤5‰ will use freshwater as the overlying water while all samples having a pore water salinity of >5‰ will use an overlying water of 10‰.

5.4 Study Conduct

5.4.1 The animals are exposed for 28 days to the test sediment and to the untreated control sediment, under static renewal conditions. Overlying water renewal is equal to two (2) volume additions per day.

5.4.2 A representative sample is obtained from sediment provided. Test sediments are homogenized and 100 mL placed in each test chamber. Sediments may be dry-sieved through a ≥3 mm screen to remove debris. 225 mL of water is added to the test chamber and allowed to settle overnight. Aeration is provided to each test chamber if the dissolved oxygen levels are <2.5 mg/L. Aeration is designed to provide approximately 1 bubble/second and set so as to not disturb the sediment surface. Prior to the addition of the test organisms, any floating detritus is removed from the surface of the water.

5.4.3 Overlying water.

5.4.3.1 Overlying water for samples having a pore water salinity of ≤5‰ will be moderately hard reconstituted laboratory water prepared as specified in Table 1 (page 26) of EPA-600/4-91/002 mixed with a natural surface water

on a 50/50 basis. Surface water will be filtered, 100µm, prior to addition to the reconstituted water. Conductivity of the overlying water will be documented and reported on a daily water quality sheet prior to use in the assay.

5.4.3.2 Overlying water for samples having a pore water salinity of >5‰ with be saltwater having a salinity of 10‰. The 10‰ saltwater will be prepared by diluting natural saltwater with deionized water. Natural saltwater will be filtered, 100 µm, prior to dilution. Salinity and conductivity of the overlying water will be documented and reported on a daily water quality sheet prior to use in the assay.

5.4.3.3 Daily overlying water renewals will be made using a systems developed by ESI. The system provides equal flow to a specified number of test chambers, variable based on test chamber size. Flow to each chamber is controlled by fixed diameter orifice. The total amount of overlying water to be exchanged is computed and added to the unit. Flow starts when water is added to the system on Day -1. Water is added to the system so that all water required for the renewal is added within 15 seconds. If the system can not hold sufficient water for the 2 volume additions then a second addition to each chamber will be made. Flow rates will be set prior to the start of an assay and maintained constant throughout the assay. Flow rates will be set to minimize disturbance of and re-suspension of the sediment surface and so as not to cause water levels to exceed that of the overflow in the test chamber.

5.4.4 Test organisms will be 7 - 8 days old at the start of the assay.

5.4.5 Each treatment group will consist of 80 animals, 10 animals per test vessel. The animals are randomly assigned to the test vessels on day 0.

5.4.6 Measurement of Water Characteristics

5.4.6.1 Prior to renewal, temperature, dissolved oxygen, specific conductance, salinity and pH are measured daily during the test, in a surrogate test chamber for each treatment. Surrogate chambers will be treated in the same manner as all other replicates and will receive test organisms and will be fed on the same schedule.

5.4.6.2 Temperature is recorded hourly using a data-logger placed in a separate sealed test chamber containing dry control sediment.

5.4.6.3 Alkalinity, hardness and ammonia of the overlying water are measured in the overlying water of a surrogate test chamber for each treatment at the start of the test, and weekly thereafter. TOC of the overlying water is measured in a surrogate at the start and end of the assay. Pore water salinity, pH, ammonia, hardness and alkalinity will be measured in a subsample of each sediment sample prior to the start of the assay.

5.4.6.4 At the end of the assay, pore water ammonia levels will be determined for each sample treatment. Pore water for this analysis will be collected from the surrogate test chamber.

5.4.7 Amphipods are fed 1.0 mL of YCT food per vessel, solids content 1800 mg/L, daily. Feeding will be suspended if fungus is noted forming on more than approximately 25% of the sediment surface.

5.4.8 The assay is terminated on day 28. Sediments are placed on an appropriately sized screen and gently washed with fresh water to remove sediment. Material remaining on the screen is examined to recover remaining amphipods. Any amphipod that shows signs of movement is considered alive, and counted.

5.4.9 All surviving amphipods from an individual replicate are rinsed with lab water, to remove any detritus, placed on a tared pan and dried at 60°C for 24 hours. Pans are allowed to cool to room temperature in a desiccator and weighed to the nearest 0.01 mg.

6.0 Quality Control Requirements

6.1 Reference Toxicant Evaluation

A reference toxicant evaluation should be conducted with each series of assays, ESI SOP#1114-R3-2003. The reference toxicant shall be a 96-hour 'water only' test conducted with cadmium chloride. A separate reference toxicant will be conducted for organisms maintained in each water type, fresh and salt.

6.2 Interferences

Living organisms present in the sample may compete with the amphipods or may be predators, reducing overall survival. This impact may be mitigated by sieving samples prior to testing.

7.0 Calculations/Reporting

7.1 Data Analysis

Survival and growth data from each treatment will be subjected to analysis of variance (ANOVA) to determine if significant differences exist between treatments and the control. Statistical evaluations will be made with CETIS® software using appropriate standard statistical models.

7.1.1 Prior to statistical analysis, the survival and growth data will be reviewed to determine the presence of outliers. If outliers are found, an explanation must be sought. If a reasonable source for the deviation is found, the value may be excluded from further analysis. If no explanation is found, the analysis should be performed both with and without the questionable data point and both sets of results reported. All data sets are evaluated to determine sample variance homogeneity and normality. Those data sets meeting the criteria for normality and homogeneity are evaluated with parametric statistical models, while those that do not meet both criteria are evaluated using non-parametric models.

7.1.2 Endpoints evaluated are: survival and growth (dry weight) at day 28.Growth endpoints evaluated include both dry weight and dry biomass. Dry weight is determined as the total dry weight divided by the number of surviving/recovered organisms. Dry biomass is determined as total dry weight divided by the number of organisms used per replicate at the start of the assay.

7.1.3 Statistical comparisons for each sample site will be made against the laboratory control treatment plus each project reference site or as otherwise specified. Project reference sites may also be compared to one another.

7.2 Reporting

Reports generated from this study will include: summarization of collection and transportation information (as provided), methods and materials, test organism history, test conditions, documentation of variations from the proposed work scope, and results and data analysis. Copies of all statistical printouts and raw data are attached as an appendix to the report.

8.0 Corrective Actions

8.1 Acceptability Criteria

8.1.1 The amphipod assay is considered acceptable if environmental parameters (temperature, dissolved oxygen, salinity, pH, alkalinity and hardness) fall within the ranges specified. Survival in the control sediments after 28 days will be ≥80%.

8.1.2 Criteria specified in Section 11 will be met.

8.2 If survival fails to meet the minimum value specified by the protocol, the client will be notified and the test restarted.

8.3 If water quality values fall outside study limits the Laboratory Manager, using sound scientific practice, will determine if the study requires repeating or the data is allowed to be accepted. The client will be notified, the results reviewed and a final determination made as to the acceptability of the data.

8.4 In the event that an element of the assay falls outside acceptable limits, or there is a change in the protocol, a Corrective Action Report must be initiated and completed.

9.0 Health and Safety

9.1 As with all samples, gloves and safety glasses should be worn when handling sediment samples and chemicals. It is advisable to wear a lab coat to protect clothing.

9.2 At the end of an assay excess sample material and material used in the assay will be disposed of appropriately. Material may be returned to the client, or air dried and placed in an appropriate container for disposal at an approved disposal facility. If the material is classified as non-hazardous, the material may be disposed in an appropriate waste container.

9.3 Assays and sample disposal will be conducted using procedures to minimize potential pollution of surface and ground waters, soils and sediments.

10.0 Responsibilities

10.1 It is the Lab Manager's responsibility to ensure analysts performing this procedure are properly trained and the training is documented in their training file. The analyst is responsible for following the procedures outlined in this SOP.

10.2 Prior to any staff member working unsupervised on a testing procedure, they must be certified by the Laboratory Manager. Certification will include reading this and associated SOPs, review of the primary literature and participation in similar procedures under the direct supervision of a trained staff member. Certification will be based upon a review of the persons' demonstrated abilities.

11.0 Summary of Test Conditions

1.	Test Mode:	Static renewal
2.	Overlying Water Renewal:	2 Volume additions per day
3.	Temperature:	Mean 23 ±1°C with no values exceeding limits of ±3°C of the mean
4.	Photoperiod:	16 hr light:8 hr dark
5.	Light Source:	Wide-spectrum fluorescent
6.	Light Intensity:	Approximately 400 to 600 Lux
7.	Test Chamber:	400 mL beakers
8.	Test Solution Volume:	100 mL sediment; 225 mL overlying water
9.	Organisms/Chamber:	10
10.	Replicates/Treatment:	8
11.	Organisms per Treatment:	80
12.	Age of Organisms:	7 - 8 days
13.	Food Source:	YCT mix; 1800 mg/L solids
14.	Feeding Regime:	1.0 mL YCT daily
15.	Overlying Water:	For samples having a pore water salinity of ≤5‰ a mix of moderately hard synthetic water (hardness of 40 - 100 mg/L, alkalinity of 20 - 70 mg/L) and natural surface water. Hardness and alkalinity of overlying water shall not vary by 50% during the assay. For samples having a pore water of >5‰ the overlying water will be 10‰ salt water
16.	Aeration:	1 bubble/second, if dissolved oxygen falls below 2.5 mg/L
17.	Test Duration:	28 Days
18.	Endpoint:	Mortality and growth (dry weight to 0.01 mg and/or length to 0.01 mm)
19.	Sample Holding Time:	8 weeks at 2-4°C after collection from the field
20.	Acceptability:	Control survival equal to or exceeding 80% and measurable growth in control treatment. Hardness, and alkalinity of overlying water not to vary by 50% during assay
21.	Analytical Support:	Daily measurement of temperature, dissolved oxygen, specific conductance, salinity and pH in overlying water of one replicate of each treatment. Measurement of alkalinity, hardness and ammonia in overlying water of one replicate of each treatment at the start and end of the assay plus weekly during the assay. Measurement of salinity, pH, ammonia, hardness and alkalinity in water isolated from a sub-sample of whole sediment prior to the start of the assay. Measurement of ammonia in pore water isolated from sediment in the surrogate test chamber at the end of the assay. Hourly temperature measurement in a separate test vessel

12.0 Organism Culture - 10‰ Salinity

Hyalella azteca will be acclimated to 10‰ salinity prior to use in assays requiring elevated overlying water salinity. Amphipod cultures will be maintained at the 10‰ salinity for a minimum of 1 month prior to use in the assays. Guidance for salinity adjustment and salinity tolerance was obtained, in part, from Chris Ingersoll. Dr. Ingersoll is the Branch Chief, Toxicology, at the U.S. Geological Survey Columbia Environmental Research Center. Based on communications with Dr. Ingersoll, the following acclimation and culture protocol will be responsive to the requirements of this program. Historical data from work conducted by the Columbia Environmental Research Center staff indicate that *H. azteca* cultured at 10‰ exhibited no negative responses when used in short-term exposure assays.

12.1 Acclimation

12.1.1 Acclimation from freshwater, culture water of<0.5‰ salinity, to 10‰ should be at a rate of salinity change of no more that 2‰ per day.

12.1.2 Salinity shall be adjusted by the addition of natural seawater.

12.1.3. Salinity will be measured using a refractometer or salinometer.

12.1.4 Daily acclimation will be maintained.

12.2 Culture

12.2.1 Once at the appropriate salinity, 10‰, organism culture techniques will follow standard protocol with respect to feeding, culture renewal and general maintenance.

12.2.2 Salinity will be measured on a regular basis as part of routine culture maintenance.

12.3 Additional Support

12.3.1 Once the cultures have become acclimated to the target salinity a series of reference toxicant assays will be conducted to establish a database for the acclimated organisms.

12.3.2 Once the cultures are of sufficient age, they will be examined weekly to establish that reproduction is occurring.

12.3.3 When a culture is started, a representative group of amphipods will be randomly selected and their dry weight determined. After 28-days, a group of adults will be removed from the culture for the purpose of obtaining dry weights. Data will be evaluated to determine that growth has occurred and compared to historical growth data associated with routine cultures.

Reference

C. Ingersoll. 2009. Personal communication. Branch Chief, Toxicology, U.S. Geological Survey Columbia Environmental Research Center.

TITLE: Acute Toxicity of Sediments To Midge Larvae, *Chironomus dilutus* - Project Specific Document

Approved By:

QA Officer:_____ Date:_____

President:_____ Date:_____

Revision History:

Revision Number	Changes	Revised By	Date
1	Review and Update	K. A. Simon	01/93
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4	Review and Update	K. A. Simon	05/98
5	Review and Update	Petra Karbe	10/99
6	Update based on new ASTM protocol	K. A. Simon	04/00
7	Update to include NELAPC material and correct light intensity	K. A. Simon	05/01
8	Review and Update, Addition of NELAC Requirements	S. Dionne	03/02
9	Update and Review	K. A. Simon	04/04
10	Update and Review	K. A. Simon	07/06
11	Update and Review	K. A. Simon	03/08
12	Update	R.A. McIsaac	01/09
12a	Project Specific Document with modifications based on comments by Dr. Chris Ingersoll, USGS, to the 28-day *Hyalella azteca* SOP that generally apply to sediment bioassay testing.	N. Roka	10/14/09
12b	Editorial corrections	R. A. McIsaac	10/15/09
12c	Revised based on comments from USEPA and CPG (Cooperating Parties Group) for the Lower Passaic River Restoration Project	K. Simon	11/18/09

TITLE: Acute Toxicity of Sediments To Midge Larvae, *Chironomus dilutus* - Project Specific Document

1.0 Purpose and Applicability

The purpose of this Standard Operating Procedure is to determine the impact, based on survival and growth, of sediments to midge larvae exposed under static renewal conditions. The exposure period for this assay is 10 days. The assay is completed using guidelines developed by ASTM and U.S. Environmental Protection Agency (USEPA) and is provided in *Standard Test Methods for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates* (ASTM E 1706-05e) and *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates*. Second Edition (USEPA).

At the end of the 10-day exposure period the midge larvae are recovered, enumerated and dried to establish survival and growth. Growth is expressed as the average weight/surviving individual and average biomass (total biomass in a replicate divided by the number of organisms in that replicate at the start of the exposure).

This document has been modified to meet project work scope requirements for the Lower Passaic River Ecological Risk Assessment. The work is being conducted under contract to Windward Environmental, LLC.

2.0 Definitions

Overlying Water: Water placed over sediment in a test chamber during a test.

Reference-Toxicity Test: A test conducted with reagent-grade reference chemical to assess the sensitivity of the test organisms. Deviations outside an established normal range may indicate a change in the sensitivity of the test organism population. Reference-toxicity tests are most often performed in the absence of sediment.

Sediment: Particulate material that usually lies below water. Formulated particulate material that is intended to lie below water in a test.

Whole Sediment: Sediment and associated pore water which have had minimal manipulation. The term bulk sediment has been used synonymously with whole sediment.

Pore Water: Water located in the spaces between grains of sediment

3.0 Applicable Documents/References

ASTM. 2009. *Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates.* E 1706-05e, West Conshohocken, PA.

U.S. EPA. 2000. *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates*. Second Edition. EPA/R-99/064. March 2000.

ESI SOP# QA-1203-R5-2003: Preparation of Daphnia Food

ESI SOP#QA-1339-R4-2008: Collection of Sediment Pore Water Samples

ESI SOP#QA-1219-R0-2009: Use and Operation of the YSI Model 556 Multi Probe System meter

ESI SOP#QA-1114-R3-2003: Conduct of Reference Toxicant Assays

ESI SOP#QA-1341-R1-2009: Sulfide Analysis by Titration

ESI SOP#QA-1320-R6-2009: Statistical Analysis of Acute and Chronic Exposure Bioassay Data

ESI SOP#QA-1309-R4-2009: Computation of Hardness by Calculation Method

ESI SOP#QA-1326-R6-2009: Alkalinity by Lachat using the Automated Phenate Method

ESI SOP#QA-1325-R8-2009: Ammonia by Lachat

ESI SOP#QA-1336-R4-2007: Measurement of Total Organic Carbon using the Phoenix 8000 Analyzer

4.0 Materials and Apparatus

Test animals of appropriate age
Beakers, 400 mL, drilled and screened
Incubator/ waterbath, capable of maintaining a temperature of 23 ±˚C
Dissolved oxygen meter, pH meter, conductivity meter, temperature logger
Light meter
Balance capable of reading 0.01 mg
Drying Oven, 60 ±5˚C
Muffle furnace, 550˚C
Tetramin® Fish Food
Components for artificial sediment - fine sand and organic material
Sieves
Refractometer
Inert gas - Nitrogen or Argon for sample storage

5.0 Methods/Procedures

5.1 Test Material

5.1.1 Test substances will be clearly identified as to source, collection date, and period of collection. A description of collection methods may also be provided. The laboratory will identify sample storage procedures and maintain receipt, storage, usage and disposition records.

5.1.2 Project site sediments are stored at 2-4˚C in the original shipping container. Maximum holding time is 8 weeks. Once open, headspace within a sample container with remaining sample is purged using an inert gas prior to storage. Inert gas can be either nitrogen or argon.

5.1.3 Prior to test preparation, the sediment is warmed to 23˚C.

5.1.4 Prior to use in the assay, sediment total organic content (loss on ignition) is measured for each sediment sample.

5.1.5 Prior to use in the assay, pore water salinity is measured using a refractometer or salinometer, as appropriate.

5.1.6 Prior to use in the assay, pore water ammonia, pH, hardness and alkalinity is measured in a sub-sample of each sediment sample.

5.2 Test Organisms

5.2.1 Healthy larvae from the same source and age, second to third instar, with at least 50% of the organisms having achieved third instar stage, are used in the tests. Midge larvae are considered to be at the third instar stage between 8 and 10 days after the egg masses hatch.

5.2.2 Confirmation of species is provided by the supplier. If not provided, ESI will use the services of a qualified taxonomist to confirm the species (e.g., the zoology department of the University of New Hampshire, the taxonomy group of Normandeau Associates, Bedford, NH). Organisms maintained by ESI will be from cultures of the confirmed species.

5.2.3 Pretest observation data concerning the source, handling procedures, disease treatment (if any), health, feeding and mortality of test animals are recorded and reported.

5.2.4 Initial weight is obtained on each batch of organisms tested by randomly selecting a minimum of 20 organisms from the pool to be used in the assay. The organisms are rinsed, placed on tared weigh pans and dried at 60˚C for 24 hours. The dried organisms are then cooled in a dessicator and weighed to the

nearest 0.01 mg to obtain mean initial dry weight.

5.3 Exposure Conditions

Exposure conditions and monitoring requirements detailed in the following paragraphs are the minimum requirements specified by ESI for this assay. Notify the project manager prior modifying exposure conditions or monitoring requirements.

5.3.1 Sediments are placed in vessels, overlying water is added and the samples are allowed to settle overnight before organisms are added to the test.

5.3.2 Assays are conducted in a static renewal mode. Overlying water is renewed at the rate of two (2) volume additions per day.

5.3.3 Mean water temperature is maintained at 23 $\pm$1˚C. The daily mean temperature must be within ±1˚C of the specified temperature and maximum temperature deviations should not exceed ±3˚C of the specified value at any time.

5.3.4 The photoperiod is set for 16 hours light : 8 hours dark. Light intensity is 400 - 1000 lux from wide spectrum fluorescent fixtures.

5.3.5 The test vessels are 400 mL beakers containing 100 mL of sediment and 225 mL of water.

5.3.6 Sediment for the laboratory control treatment is a formulated sediment. Formulated sediments are prepared, by weight, as follows:

Fine grained sand - 95%
Organic matter - 5% (approximate)

Sand used consists of an equal mix of F-75 and F-65 unground quartz (silica) sand provided by New England Silica.

The preferred source for organic matter in the artificial sediment is the organic matter recovered from either midge larvae or amphipod cultures. The material is screened to remove large matter and then autoclaved for a half hour to ensure sterility. Organic content of the control sediment will contain approximately 5% organic matter to be similar in organic content to project site sediments based on historical data for surface sediments collected from the study area between 2005 and 2008 (information provided by the CPG).

5.3.7 Overlying water is natural surface water collected by ESI from a location with a documented history of acceptable use in culturing and conducting tests with *C. dilutus*. The natural surface water is filtered using a 100µm screen prior to use. Basic properties of the overlying water, such as alkalinity, hardness, ammonia and specific conductance should not vary by more than 50% between the start and end of the assay.

5.4 Study Conduct

5.4.1 Test organisms are exposed to the test sediment(s) and untreated control sediment for 10 days, under static renewal conditions.

5.4.2 A representative sample will be obtained from the homogenized test sediment and 100 mL of sediment is placed in the test chamber. Then 225 ml of overlying water is added to the test chambers and allowed to settle overnight. Prior to the addition of the test organisms any floating detritus is removed from the surface of the water.

5.4.3 Overlying water

5.4.3.1 Overlying water is natural surface water (filtered using a 100µm screen). Conductivity of the overlying water is documented and reported on a daily water quality sheet prior to use in the assay.

5.4.3.2 Daily overlying water renewals are conducted using a system developed by ESI. The system provides equal flow to a specified number of test chambers, variable based on test chamber size. Flow to each chamber is controlled by fixed diameter orifice. The total amount of overlying water to be exchanged is computed and added to the unit. Flow starts when water is added to the system on Day -1. Water is added to the system so that all water required for the renewal is added within 15 seconds. If the system cannot hold sufficient water for the 2 volume additions then a second addition to each chamber will be made. Flow rates will be set prior to the start of an assay and maintained constant throughout the assay. Flow rates will be set to minimize disturbance and re-suspension of the sediment surface and to prevent water levels from overflowing the test chambers.

5.4.4 Test organisms are second to third instar, with 50% of the organisms having reached the third instar stage, at the start of the assay. Prior to use, test organisms should be held for a minimum of two hours under similar conditions to those used in the assay.

5.4.5 Each treatment group will consist of 80 animals with 10 animals placed randomly in each test vessel.

5.4.6 Measurement of Water Characteristics

5.4.6.1 Prior to renewal, dissolved oxygen, pH, conductivity and temperature are measured daily during the test in one surrogate test chamber for each treatment. Surrogate chambers are treated in the same manner as all other replicates including the addition of test organisms and feeding schedule.

5.4.6.2 Temperature in the water baths used to maintain constant temperature in the test chambers is measured on an hourly basis using a data-logger placed in a test chamber containing dry sediment. The chamber is weighted to keep it from floating in the water bath.

5.4.6.3 Alkalinity, hardness and ammonia are measured in the overlying water in a surrogate test chamber for each treatment at the start and end of the assay.

5.4.6.4 At the end of the assay, pore water ammonia and pH levels are determined for each sample treatment. Pore water for these measurements are collected from a surrogate test chamber.

5.4.7 Midge larvae are fed 1.5 mL of 6.0 g/L Tetramin® flake fish food suspension daily. Feeding is suspended if fungus is noted forming on more than approximately 25% of the sediment surface.

5.4.8 The assay is terminated on Day 10 at the conclusion of the full 10-day exposure period. Test sediment is removed from the test vessel, placed on an appropriately sized screen and washed with water. Midge larvae are rinsed, collected, counted, and set aside for determination of survival and growth (ash free dry weight).

5.4.9 Surviving larvae from each individual replicate are rinsed again with lab water, to remove any detritus, then placed on a tared pan and dried at 60˚C for 24 hours. Pans are cooled to room temperature in a desiccator and weighed to the nearest 0.01 mg. (This is recorded as dry weight.) Pans are then placed in a muffle furnace and organisms are ashed for two hours at 550˚C. Pans are cooled to room temperature in a desiccator and weighed to the nearest 0.01 mg. (This is recorded as ash weight.) The tissue mass of the larvae is determined as the difference between the weight of the dried larvae plus pan and the weight of the ashed larvae plus pan.

6.0 Quality Control Requirements

6.1 Acceptance Criteria

6.1.1 Mean control survival $\geq$70%, mean ash free dry weight $\geq$0.48 mg per individual. Hardness, alkalinity and ammonia levels of overlying water stock not to vary by 50% during assay

6.1.2 All criteria from Section 11: Summary of Test Conditions, must be met.

6.2 Reference Toxicant Evaluation

A reference toxicant evaluation should be conducted with each series of assays, ESI SOP#1114-R3-2003. The reference toxicant shall be a 96-hour 'water only' test

conducted with cadmium chloride.

6.3 Interferences

Living organisms present in the sample may compete with the midge larvae or may be predators, reducing overall survival.

7.0 Calculations/Reporting

7.1 Data Analysis

Survival and growth data from each treatment will be subjected to analysis of variance (ANOVA) to determine if significant differences exist between treatments and the control. Statistical evaluations will be made with CETIS® software using appropriate standard statistical models.

7.1.1 Prior to statistical analysis, the survival and growth data are reviewed to determine the presence of outliers. If outliers are found, an explanation must be sought. If a reasonable source for the deviation is found, the value may be excluded from further analysis. If no explanation is found, the analysis should be performed both with and without the questionable data point and both sets of results reported. All data sets are evaluated to determine sample variance homogeneity and normality. Those data sets meeting the criteria for normality and homogeneity are evaluated with parametric statistical models, while those that do not meet both criteria are evaluated using non-parametric models. See SOP QA-1320 - “Statistical Analysis of Acute and Chronic Exposure Bioassay Data”

7.1.2 Endpoints to be evaluated include; survival and growth, measured as ash free dry weight and ash free dry biomass, after10 days of exposure.

7.1.3 Statistical comparisons for each sample site will be made against the laboratory control treatment.

7.2 Reporting

Reports generated include: summarization of collection and transportation information (as provided), methods and materials, test organism history, test conditions, documentation of variation from proposed work scope, and results and data analysis. Copies of all statistical printouts and raw data will be attached as appendices to the report.

8.0 Corrective Actions

8.1 Acceptability Criteria. The midge assay is considered acceptable if:

8.1.1 Environmental parameters (temperature, dissolved oxygen, conductivity, pH, alkalinity and hardness) fall within the ranges specified. (i.e., hardness and alkalinity of overlying water stock does not vary by 50% during the assay).

8.1.2 Mean control survival is $\geq$70%

8.1.3 Mean ash free dry weight is $\geq$0.48 mg per individual in the controls.

8.1.4 Criteria specified in Section 11 are met.

8.2 If survival fails to meet the minimum value specified by the protocol the client will be notified and the test restarted. Water quality data are reviewed when collected and any necessary steps are taken to ensure that values approaching, or outside study limits, are corrected. In such cases the Project Manager will be notified.

8.3 If water quality values fall outside study limits the Project Manager, using sound scientific practice, will determine if the study requires repeating or the data are allowed to be accepted. The client will be notified, the results reviewed and a final determination made as to the acceptability of the data.

8.4 In the event that an element of the assay falls outside acceptable limits, or there is a change in the protocol, a Corrective Action Report must be initiated and completed.

9.0 Health and Safety

9.1 As with all samples, gloves and safety glasses should be worn when handling sediment samples and chemicals. It is advisable to wear a lab coat to protect clothing.

9.2 At the end of the process, excess sample material and material used in the collection process will be disposed of appropriately. Material may be returned to the client, or air dried and placed in an appropriate container for disposal at an approved disposal facility. If the material is classified as non-hazardous, the material may be disposed in an appropriate waste container.

9.3 Sample disposal will be conducted using procedures to minimize potential pollution of surface and ground waters, soils and sediments.

10.0 Responsibilities

10.1 It is the Project Manager's responsibility to ensure analysts performing this procedure are

properly trained and the training is documented in their training file. The analyst is responsible for following the procedures outlined in this SOP.

10.2 Prior to any staff member working unsupervised on a testing procedure, they must be certified by the Project Manager. Certification will include reading this and associated SOP's, review of the primary literature and participation in similar procedures under the direct supervision of a trained staff member. Certification will be based upon a review of the persons' demonstrated abilities.

11.0 Summary of Test Conditions

1.	Test Mode:	Static renewal
2.	Water Renewal:	2 volume additions per day
3.	Temperature:	Mean 23 ±1˚C , no values exceeding limits of ±3˚C of the mean
4.	Photoperiod:	16 hr light : 8 hr dark
5.	Light Source:	Wide-spectrum fluorescent
6.	Light Intensity:	Approximately 400 -1000 lux
7.	Test Chamber:	400 mL beakers
8.	Test Solution Volume:	100 mL sediment; 225 mL overlying water
9.	Organisms/Chamber:	10
10.	Replicates/Treatment:	8
11.	Organisms per Treatment:	80
12.	Age of Organisms:	Second to third instar with ≥50% of the midge larvae at third instar
13.	Food Source:	6.0 g/L Tetramin® flake fish food suspension
14.	Feeding Regime:	1.5 mL of flake fish food suspension per day
15.	Dilution Water:	Moderately hard reconstituted laboratory water prepared as specified in Table 1 (page 26) of EPA-600/4-91/002 mixed with a natural surface water on a 50/50 basis.
16.	Aeration:	If dissolved oxygen falls below 2.5 mg/L, then all vessels are aerated at 1 bubble/second delivered 2 cm above the sediment interface.
17.	Test Duration:	10 days
18.	Endpoint:	Mortality and growth, as ash free dry weight and ash free dry biomass (to 0.01 mg)
19.	Sample Holding Time:	8 weeks at 2-4˚C after collection from the field. Displace headspace with inert gas after opening sample container.
20.	Acceptability:	Control survival equal to or exceeding 70%; mean ash free dry weight of at least 0.48 mg per individual; hardness, alkalinity and ammonia of overlying water not to vary by more than 50% during assay.
21.	Analytical Support:	Daily measurement of temperature, dissolved oxygen, specific conductance, salinity and pH in overlying water of one replicate of each treatment. Measurement of alkalinity, hardness and ammonia in overlying water in one replicate of each treatment at the start and at the end of the assay. Measurement of salinity, ammonia, pH, hardness and alkalinity in pore water isolated from a sub-sample of whole sediment from each treatment at the start of the assay. Measurement of ammonia and pH in pore water isolated from sediment in the surrogate test chamber for each treatment at the end of the assay. Hourly temperature measurement in a separate test vessel.

TITLE: Acute Toxicity of Sediments to the Marine Amphipod, *Ampelisca abdita* - Project Specific Document

Approved By:

QA Officer:_____ Date:_____

President:_____ Date:_____

Revision History:

Revision Number	Changes	Revised By	Date
1	Update and Review	Petra Karbe	10/99
2	Review and Update	S. Dionne	06/00
3	Revise sections 5, 7 and 11	S. Dionne	02/01
4	Review and Update, Addition of NELAC Requirements	L. Hauthaway	03/02
5	Review and Update	A. Planz	07/03
6	Update	K. A. Simon	10/04
7	Update References	K. A. Simon	01/09
8	Update	R. A. McIsaac	04/09
8a	Project Specific Document with modifications based on comments by Dr. Chris Ingersoll, USGS, to the *Hyalella azteca* SOP that generally apply to sediment bioassay testing.	N. Roka	10/15/09
8b	Modified to meet additional project specific requirements	R. A. McIsaac	10/29/09
8c	Revised based on comments from USEPA and CPG (Cooperating Parties Group) for the Lower Passaic River Restoration Project	K. Simon	11/19/09

TITLE: Acute Toxicity of Sediments to the Marine Amphipod, *Ampelisca abdita* - Project Specific Document

1.0 Purpose and Applicability

This Standard Operating Procedure describes the method used for assessing the toxicity potential of marine and estuarine sediments to the amphipod, *Ampelisca abdita*. The assay is conducted using guidelines developed by ASTM and is provided in *Standard Guide for Measuring the Toxicity of Sediment-Associated Contaminants with Marine and Estuarine Amphipods.* (ASTM E1367-03).

The 10-day assay involves exposing organisms to solid phase sediment samples and overlying water in aerated 1L glass test chambers under static renewal conditions. Water quality data are collected daily, and observed sublethal effects such as inability to burrow are recorded. At the end of the 10 day exposure period, the amphipods are recovered from sediment and counted.

This document has been modified to meet project work scope requirements for the Lower Passaic River Ecological Risk Assessment. The work is being conducted under contract to Windward Environmental, LLC.

2.0 Definitions

Overlying Water: the water placed over sediment in a test chamber during a test.

Reference-Toxicity Test: a test conducted with reagent-grade reference chemical to assess the sensitivity of the test organisms. Deviations outside an established normal range may indicate a change in the sensitivity of the test organism population. Reference-toxicity tests are most often performed in the absence of sediment.

Pore water: water located in spaces between grains of sediment.

Sediment: particulate material that usually lies below water; formulated particulate material that is intended to lie below water in a test.

Whole Sediment: sediment and associated pore water which have had minimal manipulation. The term bulk sediment has been used synonymously with whole sediment.

3.0 Applicable Documents/References

U.S. EPA. 1994. *Methods for Assessing the Toxicity of Sediment-associated Contaminants with Estuarine and Marine Amphipods*. EPA 600/R-94/025.

ASTM. 2008. 11.06, *Standard Guide for Measuring the Toxicity of Sediment-Associated Contaminants with Marine and Estuarine Amphipods*. E-1367-03 (2008).

ESI SOP# QA-1203-R5-2003: Preparation of Daphnia Food

ESI SOP#QA-1339-R4-2008: Collection of Sediment Pore Water Samples"

ESI SOP#QA-1219-R0-2009: Use and Operation of the YSI Model 556 Multi Probe System meter

ESI SOP#QA-1114-R3-2003: Conduct of Reference Toxicant Assays

ESI SOP QA-1118-R3-2006: Corrective Action Reports

ESI SOP#QA-1341-R1-2009: Sulfide Analysis by Titration

ESI SOP#QA-1320-R6-2009: Statistical Analysis of Acute and Chronic Exposure Bioassay Data

ESI SOP#QA-1309-R4-2009: Computation of Hardness by Calculation Method

ESI SOP#QA-1326-R6-2009: Alkalinity by Lachat using the Automated Phenate Method

ESI SOP#QA-1325-R8-2009: Ammonia by Lachat

ESI SOP#QA-1336-R4-2007: Measurement of Total Organic Carbon using the Phoenix 8000 Analyzer

4.0 Materials and Apparatus

A. abdita; immature, 2-4mm total length
Beakers, 1L, drilled and screened to facilitate water exchanges
Incubator/water bath capable of maintaining a temperature of 20 ±1˚C
Aeration system
Natural control sediment
Natural sea water, 30ppt
Dissolved oxygen meter, pH meter, conductivity meter, salinity meter and temperature logger
Light table
Light meter
Sieves
Refractometer

5.0 Methods/Procedures

5.1 Test Material

5.1.1 Test substances will be clearly identified as to their source, collection date, and period of collection. A description of collection methods may also be provided. This information must be recorded on a Chain of Custody record which accompanies the samples, unless arranged otherwise. Sample handling (i.e. receipt, storage and disposition) will be recorded and maintained by ESI personnel. See SOP QA-1109 – “Sample Receipt, Handling and Disposal” for more information.

5.1.2 Project site sediments are stored at 2-4˚C in the original shipping container. The maximum holding time for the sediment is eight (8) weeks. Once open and prior to restorage, headspace within a sample container is purged using an inert gas. Inert gas can be either nitrogen or argon.

5.1.3 Prior to test preparation, the sediment is warmed to test temperature (20˚C).

5.1.4 Prior to use, sediment total organic content (loss on ignition) is measured for each sediment sample.

5.1.5 Prior to use in the assay, pore water salinity will be measured using a refractometer or salinometer, as appropriate.

5.1.6 Prior to use in the assay, sediment pore water ammonia and pH will be measured.

5.2 Test Species

5.2.1 Amphipods used in testing are immature, 3-5 mm in total length. No adult males or females are used in the assay. Organisms are obtained from a commercial source which meets ESI quality assurance standards. Amphipods received from a commercial supplier are maintained under static renewal conditions, at similar water quality, photoperiod and temperature as will be used during testing.

5.2.2 Confirmation of species is provided by the supplier. If not provided, the species will be verified using appropriate taxonomic keys. ESI will use the services of a qualified taxonomist to confirm the species (e.g., the zoology department of the University of New Hampshire or the taxonomy group of Normandeau Associates, Bedford, NH). Organisms maintained by ESI will be from cultures of the confirmed species.

5.2.3 Pretest observation data concerning the source, handling procedures, disease treatment (if any), health, feeding, mean dry weight and mortality of test animals is recorded and reported.

5.3 Exposure Conditions

Exposure conditions and monitoring requirements detailed in the following paragraphs are the minimum requirements specified by ESI for this assay. Notify the project manager prior to modifying exposure conditions or monitoring requirements.

5.3.1 Test chambers are 1 liter drilled, screened beakers filled with 2 cm (~175 mL) of sediment with overlying water brought to 800 mL total volume.

5.3.2 Overlying water is natural seawater, obtained from the Hampton/Seabrook Estuary adjusted to a salinity of 30 ppt and temperature of 20 ± 1˚C. Water may be passed through a filter, prior to use, to remove debris. If the dilution water salinity is below the range of 30±2 ppt, the sample is adjusted with commercially

purchased marine salts.

5.3.3 Control sediment is a natural marine sediment obtained from the organism supplier and collected from the same location as the test organisms. The control sediment is sieved through a ≥2mm screen to remove large debris and living organisms. The source of the control sediment must be indicated the final report.

5.3.4 The assay is conducted in static renewal mode, overlying water is replaced, at a rate of two volume additions daily.

5.3.5 Dissolved oxygen concentration is maintained at >6.0 mg/L saturation during the test. Dilution water is extensively aerated to assure dissolved oxygen concentrations are above 6.0 mg/L prior to use. Test chambers are aerated from initiation at a rate of 100 bubbles/minute.

5.3.6 Photoperiod is set at 24 hours light : 0 hours dark. Light intensity is maintained at 500-1000 Lux.

5.3.7 Sediment pore water unionized ammonia levels must be analyzed before the initiation of the assay. See SOP QA-1339 – "Collection of Sediment Pore Water Samples". The unionized ammonia levels must be below 0.4 mg/L in all treatments before the start of the assay. In the event that unionized ammonia levels are above 0.4 mg/L, the affected treatments are rinsed with 2 volume additions of laboratory control dilution water daily, and ammonia values are reanalyzed until the value is within acceptable bounds.

5.4 Study Conduct

5.4.1 Prior to the initiation of the assay, sufficient sediment must be added to each test chamber to maintain the required 2 cm depth (~175mL). Sediment is smoothed to a uniform depth. The volume of sediment in the vessels must be consistent among all test treatments.

5.4.2 The appropriate amount of overlying seawater is added to the test vessels. Vessels are allowed to settle, undisturbed, for at least 24 hours prior to the addition of test organisms.

5.4.3 Each test treatment consists of 5 replicates with a total of 100 organisms, 20 amphipods per test chamber.

5.4.4 Organisms are randomly assigned to vessels (providing that unionized ammonia concentrations are less than 0.04 mg/L) as follows:

5.4.4.1 Amphipods are counted, into pill cups containing seawater. Ten organisms are added to each pill cup. Two pill cups of organisms are added to each vessel. Pill cups must again be checked, to ensure that all organisms have been added to the vessel because *A. abdita* have a tendency to stick to pipets and pill cups.

5.4.4.2 Vessels are checked one hour after organisms are added. Organisms that have not burrowed into the sediment may be replaced. Organisms that burrow, but then resurface, are not replaced. Amphipods that are caught on the surface of the overlying water are gently sunk.

5.4.5 Animals are not fed during the assay.

5.4.6. Measurement of Water Characteristics

5.4.6.1 Prior to renewal, dissolved oxygen, pH, salinity and temperature are measured daily during the test in one surrogate test chamber for each treatment. Surrogate chambers are treated in the same manner as all other replicates including the addition of test organisms.

5.4.6.2 Temperature of the water baths used to maintain constant temperature in the test chambers is measured on an hourly basis using a data-logger placed in a test chamber containing dry sediment. The chamber is weighted to keep it from floating in the water bath.

5.4.6.3 Overlying water and sediment pore water from the surrogate test chamber is analyzed for ammonia and pH at the start, day 3 and end of the assay.

5.4.7 Dead organisms should be recorded and removed daily. An amphipod is considered dead if it does not respond to gentle probing. Throughout the assay, the following organismal behaviors should be noted as they are observed: test organisms that have left their tubes on the sediment or water surface, organisms that are near-dead and only exhibiting a pleopod muscular twitch, and the presence of molts. Emergence from the sediment and the inability of the organisms to construct proper tubes are sublethal behaviors that may ultimately result in death.

5.5 Study Termination

5.5.1 The assay is terminated on Day 10 at the conclusion of the full 10-day exposure period. Water quality parameters are recorded and ammonia samples are collected following the schedule in Section 5.4.6.

5.5.2 The contents of each vessel are individually washed through an appropriately sized screen and gently rinsed with overlying control water to remove sediment. The material remaining on the screen is examined to recover remaining amphipods. Any amphipod that shows signs of movement is considered alive, and recorded.

5.5.3 *A. abdita* are tube-dwelling organisms. It is imperative that all sediment is examined very carefully. Sediment tubes and clumps must be gently broken apart to recover all surviving organisms.

6.0 Quality Control Requirements

6.1 For the assay to be considered valid, conditions must meet those specified in Section 11: "Summary of Test Conditions". The acceptability criteria requires ≥90% survival in the control sediments.

6.2 A reference toxicant test must be successfully conducted with each batch of organisms used for testing, ESI SOP#1114-R3-2003. The assay can run concurrent with a client assay. See SOP QA-1114 - “Conduct of Reference Toxicant Assays”. The reference toxicant shall be a 96-hour ‘water only’ test conducted with cadmium chloride.

6.3 It is crucial to refer to each project’s scope of work before the beginning of testing to ensure that all individual requirements are satisfied.

6.4 It is imperative that all materials (i.e. data sheets) are reviewed on a daily basis by personnel performing the assay to ensure that all applicable data are available and accurate.

6.5 Living organisms present in the sample may compete with the amphipods or may be predators, reducing overall survival.

7.0 Calculations/Reporting

7.1 Data Analysis

Survival and data from each treatment will be subjected to analysis of variance (ANOVA) to determine if significant differences exist between treatments and the control. Statistical evaluations will be made with CETIS® software using appropriate standard statistical models.

7.1.1 Prior to statistical analysis, the survival data will be reviewed to determine the presence of outliers. If outliers are found, an explanation must be sought. If a reasonable source for the deviation is found, the value may be excluded from further analysis. If no explanation is found, the analysis should be performed both with and without the questionable data point and both sets of results reported. All data sets are evaluated to determine sample variance homogeneity and normality. Those data sets meeting the criteria for normality and homogeneity are evaluated with parametric statistical models, while those that do not meet both criteria are evaluated using non-parametric models.

7.1.2 The endpoint to be evaluated is survival after 10 days of exposure.

7.1.3 Statistical comparisons for each sample site will be made against the laboratory control treatment.

7.2 Reporting

Reports generated from this study will include: summarization of collection and transportation information (as provided), methods and materials, test organism history, test conditions, documentation of variations from the proposed work scope, results and data analysis. Copies of all statistical printouts and raw data are attached as appendices to the report.

8.0 Corrective Actions

8.1 The amphipod assay is considered acceptable if survival in the control sediments after 10 days is ≥90%.

8.2 If any parameter fails to meet the criteria specified in the "Summary of Test Conditions", the laboratory manager or project director must be notified immediately.

8.3 If the results of the reference toxicant assay are outside two standard deviations of the historic laboratory mean, the laboratory manager must be notified immediately.

8.4 In the event that an element of the assay falls outside acceptable limits, or there is a change in the protocol, a Corrective Action Report must be initiated and completed. Refer to SOP QA-1118 - “Corrective Action Reports”.

9.0 Health and Safety

9.1 As with all samples, gloves and safety glasses should be worn when handling samples and chemicals. It is advisable to wear a lab coat to protect clothing.

9.2 At the end of an assay excess sample material and material used in the assay will be disposed of properly. Material may be returned to the client, or air dried and placed in an appropriate container for disposal at an approved disposal facility. If the material is classified as non-hazardous, the material may be disposed in an appropriate waste container.

9.3 Assays and sample disposal will be conducted using procedures to minimize potential pollution of surface and ground waters, soils and sediments.

10.0 Responsibilities

10.1 It is the Lab Manager's responsibility to ensure analysts performing this procedure are properly trained and the training is documented in their training file. The analyst is responsible for following the procedures outlined in this SOP.

10.2 Prior to any staff member working unsupervised on a testing procedure, they must be certified by the Laboratory Manager. Certification will include reading this and associated

SOPs, review of the primary literature and participation in similar procedures under the direct supervision of a trained staff member. Certification will be based upon a review of the analysts' demonstrated abilities.

11.0 Summary of Test Conditions

1. Test Mode:	Static Renewal
2. Test Duration:	10 Days
3. Renewal Schedule:	2 volume exchanges daily in Static Renewal Mode
4. Temperature:	20 ±1˚C
5. Photoperiod:	24 hr light / 0 hr dark
6. Light Source:	Wide - spectrum fluorescent
7. Light Intensity:	500 to1000 Lux
8. Salinity:	30±2 ppt
9. Test Chamber:	1000 mL beakers
10. Solution Volume:	800 mL overlying water
11. Sediment Depth:	2 cm (~175 mL)
12. Organisms/Chamber:	20
13. Replicates/Treatment:	5 per treatment
14. Treatments:	Site Sediment, Control Sediment
15. Age of Organisms:	Immature amphipods, 3 to 5 mm - no reproductive adults
16. Feeding Regime:	None
17. Overlying Water:	Natural Seawater
18. Aeration:	Continuous aeration to ≥6.0 mg/L
19. Endpoint:	Survival
20. Acceptability:	Mean lab control survival of ≥90%
21. Support Chemistry:	Daily measurement of dissolved oxygen, pH, salinity and temperature in a surrogate vessel for each treatment. Overlying ammonia on days 0, 3 and 10. Pore water ammonia and pH on days 0, 3, and 10. Hourly temperature readings in one surrogate vessel.
22. Sample Holding Requirements:	Storage in dark, locked refrigerator at 2-4˚C; maximum holding time is 8 weeks from date sampled. Headspace in opened sample containers with remaining sample will be purged using an inert gas prior to storage. Inert gas will be either nitrogen or argon.

TITLE: Assessment Toxicity (28-Day) of Sediments To The Amphipod, *Hyalella azteca* based on Survival and Growth -Project Specific Document. Rev. 6

Approved By:

QA Officer:_____ Date:_____

President:_____ Date:_____

Revision History:

Revision Number	Changes	Revised By	Date
0	Preparation of SOP	K. A. Simon	6/00
1	Review and update. Correction of light intensity	K. A. Simon	4/01
2	Update, NELAC revisions	K.A. Simon	07/01
3	Clerical Corrections clarifications related to water sources and sieving	K.A. Simon	09/01
4	Review and Update, Addition of NELAC Requirements	S. Dionne	03/02
5	Review and Update	K. A. Simon	04/04
6	Review and Update	K. A. Simon	07/06
7	Review and Update	R. A. McIsaac	01/09
7a	Project Specific Document	K. A. Simon	08/09
7b	Project Specific Document Rev 1	K. A. Simon	09/09
7c	Project Specific Document Rev 2. Includes modification suggest during 09/15/09 teleconference with Chris Ingersoll, USGS	K. A. Simon	09/09
7d	Project Specific Document Rev 3. Includes modification suggest during 09/28/09 teleconference with Chris Ingersoll, USGS	K. A. Simon	09/29/09
7e	Addition of pore water ammonia monitoring at the end of the assay. Rev 4	K. A. Simon	10/05/09
7f	Final EPA suggested changes to text. Rev 5	K. A. Simon	10/18/09
7g	Modification of Pore Water Monitoring Parameters in ¶ 5.4.6.3 and 11.21. Rev 6	K. A. Simon	10/22/09

1.0 Purpose and Applicability

The purpose of this Standard Operating Procedure is to determine the impact, based on survival and growth, of sediments to amphipods exposed under static renewal conditions. The assay involves exposing amphipods to a sediment over a 28 day period. The assay is conducted using guidelines developed by ASTM and is provided in *Standard Test Methods for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates* (ASTM E1706-05e).

Hyalella azteca (Saussure), Amphipoda, have many desirable characteristics of an ideal sediment toxicity testing organism including: relative sensitivity to contaminants associated with sediment, short generation time, contact with sediment, ease of culture in the laboratory, and tolerance to varying physico-chemical characteristics of sediment.

At the end of the 28 day exposure period the amphipods are recovered and counted to establish survival, then dried to establish growth expressed as the average weight/surviving individual and average biomass (total biomass in a replicate divided by the number of organisms in that replicate at the start of the exposure).

This document has been modified to meet project work scope requirements, specified by the U.S. Environmental Protection Agency, for the Lower Passaic River Ecological Risk Assessment. The work is being conducted under contract to Windward Environmental, LLC. Modifications incorporated into the document are related to the salinity of the overlying water used during the assay and culture and acclimation of the test organisms. These modifications are being made to allow the use of a single test species over an extended range of the project where salinity regimes vary beyond the normal range utilized for the species.

2.0 Definitions

Overlying Water: The water placed over sediment in a test chamber during a test.

Reference Sediment: A whole sediment near an area of concern used to assess sediment conditions exclusive of material(s) of interest. The reference sediment may be used as an indicator of localized sediment conditions exclusive of the specific pollutant input of concern. Such sediment would be collected near the site of concern and would represent the background conditions resulting from any localized pollutant inputs as well as global pollutant input. This is the manner in which reference sediment is used in dredge material evaluations.

Reference-Toxicity Test: A test conducted with reagent-grade reference chemical to assess the sensitivity of the test organisms. Deviations outside an established normal range may indicate a change in the sensitivity of the test organism population. Reference-toxicity tests are most often performed in the absence of sediment.

Pore water: Water located in spaces between grains of sediment.

Sediment: Particulate material that usually lies below water. Formulated particulate material that is intended to lie below water in a test.

Whole Sediment: Sediment and associated pore water which have had minimal manipulation. The term bulk sediment has been used synonymously with whole sediment.

3.0 Applicable Documents/References

ASTM. 2009. *Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates.* E 1706-05e, West Conshohocken, PA.

U.S. EPA. 2000. *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates.* Second Edition. EPA/R-99/064. March 2000.

ESI SOP# QA-1203-R5-2003: Preparation of Daphnia Food

ESI SOP#QA-1339-R4-2008: Collection of Sediment Pore Water Samples

ESI SOP#QA-1219-R0-2009: Use and Operation of the YSI Model 556 Multi Probe System meter

ESI SOP#QA-1114-R3-2003: Conduct of Reference Toxicant Assays

ESI SOP#QA-1341-R1-2009: Sulfide Analysis by Titration

ESI SOP#QA-1320-R6-2009: Statistical Analysis of Acute and Chronic Exposure Bioassay Data

ESI SOP#QA-1309-R4-2009: Computation of Hardness by Calculation Method

ESI SOP#QA-1326-R6-2009: Alkalinity by Lachat using the Automated Phenate Method

ESI SOP#QA-1325-R8-2009: Ammonia by Lachat

ESI SOP#QA-1336-R4-2007: Measurement of Total Organic Carbon using the Phoenix 8000 Analyzer

4.0 Materials and Apparatus

Test animals
Beakers, 400 mL, drilled and screened for flow through
Incubator/water bath capable of maintaining a temperature of 23 ±1°C
Dissolved oxygen meter, pH meter, conductivity meter, temperature logger
Light Meter
Balance, capable of reading 0.01 mg
Drying Oven, 60°C
Components for artificial sediment - fine sand, organic material
Sieves
YCT Food (See SOP #1203)
Refractometer

5.0 Methods/Procedures

5.1 Test Material

5.1.1 Test substances will be clearly identified as to source, collection date, period of collection and description of collection methods. The laboratory will identify sample storage procedures and maintain receipt, storage, usage and disposition records.

5.1.2 Project site sediments will be stored at 2-4°C in the original shipping container.

Maximum holding time is 8 weeks. Once open, a sample container with remaining sample will be purged using an inert gas prior to storage. Inert gas will be either nitrogen or argon.

5.1.3 Prior to test preparation, the sediment is warmed to 23°C.

5.1.4 Prior to use, sediment total organic content (loss on ignition) is measured for each sediment sample.

5.1.5 Prior to use in the assay, pore water salinity will be measured using a refractometer or salinometer, as appropriate. Samples having a pore water salinity of ≤5‰ will be identified as being tested using freshwater as the overlying water while all samples having a pore water salinity of >5‰ will be identified as being tested using a 10‰ salinity overlying water.

5.2 Test Organisms

5.2.1 Healthy amphipods from the same source and age are used in the test. Amphipods will be between 7 and 8 days old.

5.2.2 Confirmation of species is provided by the supplier. If not provided, ESI will use the services of either the zoology department of the University of New Hampshire or the taxonomy group of Normandeau Associates, Bedford, NH to confirm the species. Organisms maintained by ESI will be from cultures of the confirmed species.

5.2.3 Pretest observation data concerning the source, handling procedures, disease treatment (if any), health, feeding, mortality and mean dry weight of test animals will be recorded and reported. Mean dry weight of organisms at the start of the assay are based on that of 40 organisms measured in groups of 10. Growth, dry weight, of similar aged adult amphipods will be monitored in both freshwater and saltwater. Acclimated amphipod cultures will be monitored prior to use in the assays to provide a qualitative measurement of growth in the two culture systems. Young production in all cultures will be qualitatively compared to determine if there are substantial differences in reproduction between freshwater cultures and saltwater-acclimated cultures.

5.2.4 Organisms used in the assays will be selected from cultures of appropriate salinity. Salinity levels utilized will be either freshwater, <0.5‰, or 10‰, depending on the pore water salinity of an individual sample.

5.3 Exposure Conditions

Exposure conditions and monitoring requirements detailed in the following paragraphs are the minimum requirements specified by ESI for this assay.

5.3.1 Sediments are placed in vessels, overlying water is added and the samples are allowed to settle overnight before organisms are added to the test.

5.3.2 A laboratory control will be established for both fresh and salt water sample sets.

5.3.3 Assays are conducted in a static renewal mode. Overlying water is renewed on a

daily basis, at the rate of two (2) volume additions per day.

5.3.4 Mean water temperature is 23 ±1°C. Maximum temperature deviations should not exceed ±3°C of the specified value at any time.

5.3.5 The photoperiod is set to 16 hours light : 8 hours dark. Light intensity is 100 to 1000 lux from wide spectrum fluorescent fixtures.

5.3.6 The test vessels are 400 mL beakers containing 100 mL of sediment and 225 mL of water.

5.3.7 Sediment for the laboratory control treatment will be a formulated sediment. Formulated sediments will be prepared, by weight, as follows:

Fine grained sand	-	95%
Organic matter	-	5% (approximate)

Sand used will be an equal mix of F-75 and F-65 unground quartz (silica) sand provided by New England Silica

The preferred source for organic matter in the artificial sediment is the organic matter recovered from either midge larvae or amphipod cultures. The material is screened to remove large matter and then autoclaved for a half hour to insure sterility. Organic content of the control sediment will be adjusted prior to the start of the assay to be representative of that of the project site sediments.

5.3.8 The type of the overlying water will be determined based on individual sample pore salinity. Samples with a pore water salinity of ≤5‰ will use freshwater as the overlying water while all samples having a pore water salinity of >5‰ will use an overlying water of 10‰.

5.4 Study Conduct

5.4.1 The animals are exposed for 28 days to the test sediment and to the untreated control sediment, under static renewal conditions. Overlying water renewal is equal to two (2) volume additions per day.

5.4.2 A representative sample is obtained from sediment provided. Test sediments are homogenized and 100 mL placed in each test chamber. Sediments may be dry-sieved through a ≥3 mm screen to remove debris. 225 mL of water is added to the test chamber and allowed to settle overnight. Aeration is provided to each test chamber if the dissolved oxygen levels are <2.5 mg/L. Aeration is designed to provide approximately 1 bubble/second and set so as to not disturb the sediment surface. Prior to the addition of the test organisms, any floating detritus is removed from the surface of the water.

5.4.3 Overlying water.

5.4.3.1 Overlying water for samples having a pore water salinity of ≤5‰ will be moderately hard reconstituted laboratory water prepared as specified in Table 1 (page 26) of EPA-600/4-91/002 mixed with a natural surface water

on a 50/50 basis. Surface water will be filtered, 100µm, prior to addition to the reconstituted water. Conductivity of the overlying water will be documented and reported on a daily water quality sheet prior to use in the assay.

5.4.3.2 Overlying water for samples having a pore water salinity of >5‰ with be saltwater having a salinity of 10‰. The 10‰ saltwater will be prepared by diluting natural saltwater with deionized water. Natural saltwater will be filtered, 100 µm, prior to dilution. Salinity and conductivity of the overlying water will be documented and reported on a daily water quality sheet prior to use in the assay.

5.4.3.3 Daily overlying water renewals will be made using a systems developed by ESI. The system provides equal flow to a specified number of test chambers, variable based on test chamber size. Flow to each chamber is controlled by fixed diameter orifice. The total amount of overlying water to be exchanged is computed and added to the unit. Flow starts when water is added to the system on Day -1. Water is added to the system so that all water required for the renewal is added within 15 seconds. If the system can not hold sufficient water for the 2 volume additions then a second addition to each chamber will be made. Flow rates will be set prior to the start of an assay and maintained constant throughout the assay. Flow rates will be set to minimize disturbance of and re-suspension of the sediment surface and so as not to cause water levels to exceed that of the overflow in the test chamber.

5.4.4 Test organisms will be 7 - 8 days old at the start of the assay.

5.4.5 Each treatment group will consist of 80 animals, 10 animals per test vessel. The animals are randomly assigned to the test vessels on day 0.

5.4.6 Measurement of Water Characteristics

5.4.6.1 Prior to renewal, temperature, dissolved oxygen, specific conductance, salinity and pH are measured daily during the test, in a surrogate test chamber for each treatment. Surrogate chambers will be treated in the same manner as all other replicates and will receive test organisms and will be fed on the same schedule.

5.4.6.2 Temperature is recorded hourly using a data-logger placed in a separate sealed test chamber containing dry control sediment.

5.4.6.3 Alkalinity, hardness and ammonia of the overlying water are measured in the overlying water of a surrogate test chamber for each treatment at the start of the test, and weekly thereafter. TOC of the overlying water is measured in a surrogate at the start and end of the assay. Pore water salinity, pH, ammonia, hardness and alkalinity will be measured in a subsample of each sediment sample prior to the start of the assay.

5.4.6.4 At the end of the assay, pore water ammonia levels will be determined for each sample treatment. Pore water for this analysis will be collected from the surrogate test chamber.

5.4.7 Amphipods are fed 1.0 mL of YCT food per vessel, solids content 1800 mg/L, daily. Feeding will be suspended if fungus is noted forming on more than approximately 25% of the sediment surface.

5.4.8 The assay is terminated on day 28. Sediments are placed on an appropriately sized screen and gently washed with fresh water to remove sediment. Material remaining on the screen is examined to recover remaining amphipods. Any amphipod that shows signs of movement is considered alive, and counted.

5.4.9 All surviving amphipods from an individual replicate are rinsed with lab water, to remove any detritus, placed on a tared pan and dried at 60°C for 24 hours. Pans are allowed to cool to room temperature in a desiccator and weighed to the nearest 0.01 mg.

6.0 Quality Control Requirements

6.1 Reference Toxicant Evaluation

A reference toxicant evaluation should be conducted with each series of assays, ESI SOP#1114-R3-2003. The reference toxicant shall be a 96-hour 'water only' test conducted with cadmium chloride. A separate reference toxicant will be conducted for organisms maintained in each water type, fresh and salt.

6.2 Interferences

Living organisms present in the sample may compete with the amphipods or may be predators, reducing overall survival. This impact may be mitigated by sieving samples prior to testing.

7.0 Calculations/Reporting

7.1 Data Analysis

Survival and growth data from each treatment will be subjected to analysis of variance (ANOVA) to determine if significant differences exist between treatments and the control. Statistical evaluations will be made with CETIS® software using appropriate standard statistical models.

7.1.1 Prior to statistical analysis, the survival and growth data will be reviewed to determine the presence of outliers. If outliers are found, an explanation must be sought. If a reasonable source for the deviation is found, the value may be excluded from further analysis. If no explanation is found, the analysis should be performed both with and without the questionable data point and both sets of results reported. All data sets are evaluated to determine sample variance homogeneity and normality. Those data sets meeting the criteria for normality and homogeneity are evaluated with parametric statistical models, while those that do not meet both criteria are evaluated using non-parametric models.

7.1.2 Endpoints evaluated are: survival and growth (dry weight) at day 28.Growth endpoints evaluated include both dry weight and dry biomass. Dry weight is determined as the total dry weight divided by the number of surviving/recovered organisms. Dry biomass is determined as total dry weight divided by the number of organisms used per replicate at the start of the assay.

7.1.3 Statistical comparisons for each sample site will be made against the laboratory control treatment plus each project reference site or as otherwise specified. Project reference sites may also be compared to one another.

7.2 Reporting

Reports generated from this study will include: summarization of collection and transportation information (as provided), methods and materials, test organism history, test conditions, documentation of variations from the proposed work scope, and results and data analysis. Copies of all statistical printouts and raw data are attached as an appendix to the report.

8.0 Corrective Actions

8.1 Acceptability Criteria

8.1.1 The amphipod assay is considered acceptable if environmental parameters (temperature, dissolved oxygen, salinity, pH, alkalinity and hardness) fall within the ranges specified. Survival in the control sediments after 28 days will be ≥80%.

8.1.2 Criteria specified in Section 11 will be met.

8.2 If survival fails to meet the minimum value specified by the protocol, the client will be notified and the test restarted.

8.3 If water quality values fall outside study limits the Laboratory Manager, using sound scientific practice, will determine if the study requires repeating or the data is allowed to be accepted. The client will be notified, the results reviewed and a final determination made as to the acceptability of the data.

8.4 In the event that an element of the assay falls outside acceptable limits, or there is a change in the protocol, a Corrective Action Report must be initiated and completed.

9.0 Health and Safety

9.1 As with all samples, gloves and safety glasses should be worn when handling sediment samples and chemicals. It is advisable to wear a lab coat to protect clothing.

9.2 At the end of an assay excess sample material and material used in the assay will be disposed of appropriately. Material may be returned to the client, or air dried and placed in an appropriate container for disposal at an approved disposal facility. If the material is classified as non-hazardous, the material may be disposed in an appropriate waste container.

9.3 Assays and sample disposal will be conducted using procedures to minimize potential pollution of surface and ground waters, soils and sediments.

10.0 Responsibilities

10.1 It is the Lab Manager's responsibility to ensure analysts performing this procedure are properly trained and the training is documented in their training file. The analyst is responsible for following the procedures outlined in this SOP.

10.2 Prior to any staff member working unsupervised on a testing procedure, they must be certified by the Laboratory Manager. Certification will include reading this and associated SOPs, review of the primary literature and participation in similar procedures under the direct supervision of a trained staff member. Certification will be based upon a review of the persons' demonstrated abilities.

11.0 Summary of Test Conditions

1.	Test Mode:	Static renewal
2.	Overlying Water Renewal:	2 Volume additions per day
3.	Temperature:	Mean 23 ±1°C with no values exceeding limits of ±3°C of the mean
4.	Photoperiod:	16 hr light:8 hr dark
5.	Light Source:	Wide-spectrum fluorescent
6.	Light Intensity:	Approximately 400 to 600 Lux
7.	Test Chamber:	400 mL beakers
8.	Test Solution Volume:	100 mL sediment; 225 mL overlying water
9.	Organisms/Chamber:	10
10.	Replicates/Treatment:	8
11.	Organisms per Treatment:	80
12.	Age of Organisms:	7 - 8 days
13.	Food Source:	YCT mix; 1800 mg/L solids
14.	Feeding Regime:	1.0 mL YCT daily
15.	Overlying Water:	For samples having a pore water salinity of ≤5‰ a mix of moderately hard synthetic water (hardness of 40 - 100 mg/L, alkalinity of 20 - 70 mg/L) and natural surface water. Hardness and alkalinity of overlying water shall not vary by 50% during the assay. For samples having a pore water of >5‰ the overlying water will be 10‰ salt water
16.	Aeration:	1 bubble/second, if dissolved oxygen falls below 2.5 mg/L
17.	Test Duration:	28 Days
18.	Endpoint:	Mortality and growth (dry weight to 0.01 mg and/or length to 0.01 mm)
19.	Sample Holding Time:	8 weeks at 2-4°C after collection from the field
20.	Acceptability:	Control survival equal to or exceeding 80% and measurable growth in control treatment. Hardness, and alkalinity of overlying water not to vary by 50% during assay
21.	Analytical Support:	Daily measurement of temperature, dissolved oxygen, specific conductance, salinity and pH in overlying water of one replicate of each treatment. Measurement of alkalinity, hardness and ammonia in overlying water of one replicate of each treatment at the start and end of the assay plus weekly during the assay. Measurement of salinity, pH, ammonia, hardness and alkalinity in water isolated from a sub-sample of whole sediment prior to the start of the assay. Measurement of ammonia in pore water isolated from sediment in the surrogate test chamber at the end of the assay. Hourly temperature measurement in a separate test vessel

12.0 Organism Culture - 10‰ Salinity

Hyalella azteca will be acclimated to 10‰ salinity prior to use in assays requiring elevated overlying water salinity. Amphipod cultures will be maintained at the 10‰ salinity for a minimum of 1 month prior to use in the assays. Guidance for salinity adjustment and salinity tolerance was obtained, in part, from Chris Ingersoll. Dr. Ingersoll is the Branch Chief, Toxicology, at the U.S. Geological Survey Columbia Environmental Research Center. Based on communications with Dr. Ingersoll, the following acclimation and culture protocol will be responsive to the requirements of this program. Historical data from work conducted by the Columbia Environmental Research Center staff indicate that *H. azteca* cultured at 10‰ exhibited no negative responses when used in short-term exposure assays.

12.1 Acclimation

12.1.1 Acclimation from freshwater, culture water of<0.5‰ salinity, to 10‰ should be at a rate of salinity change of no more that 2‰ per day.

12.1.2 Salinity shall be adjusted by the addition of natural seawater.

12.1.3. Salinity will be measured using a refractometer or salinometer.

12.1.4 Daily acclimation will be maintained.

12.2 Culture

12.2.1 Once at the appropriate salinity, 10‰, organism culture techniques will follow standard protocol with respect to feeding, culture renewal and general maintenance.

12.2.2 Salinity will be measured on a regular basis as part of routine culture maintenance.

12.3 Additional Support

12.3.1 Once the cultures have become acclimated to the target salinity a series of reference toxicant assays will be conducted to establish a database for the acclimated organisms.

12.3.2 Once the cultures are of sufficient age, they will be examined weekly to establish that reproduction is occurring.

12.3.3 When a culture is started, a representative group of amphipods will be randomly selected and their dry weight determined. After 28-days, a group of adults will be removed from the culture for the purpose of obtaining dry weights. Data will be evaluated to determine that growth has occurred and compared to historical growth data associated with routine cultures.

Reference

C. Ingersoll. 2009. Personal communication. Branch Chief, Toxicology, U.S. Geological Survey Columbia Environmental Research Center.